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Perspective on TSE Clearance Studies

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Clearance Studies 1:

Bovine albumin	brain spike
Bovine aprotinin	brain spike
Cohn Fractionation	
Fukuoka GSS	endogenous
Hamster 263K scrapie	brain cells
Hamster 263K scrapie	endogenou
Factor VIII	hamster brain
IVIG	hamster brain
Kistler-Nitschmann Fractionation	
Human albumin	hamster brain
Human albumin	fibrils
IVIG	hamster brain
IVIG	fibrils

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Clearance Studies 2:

Bovine collagen 1	brain spike
Bovine collagen 2	brain spike
Bovine gelatin	brain spike
Filtration Asahi Planova filters	brain spike
Filtration Asahi Planova filters	fibrils
Irradiation Clearant Process	brain spike
Others	

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Process steps:

- Depth filtrations
- Membrane filtrations
- Phase separations
- Extractions
- Precipitations
- Column chromatography
- Thermal inactivation
- Irradiation
- Chemical inactivation
- Others

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Key Elements:

- Scale down
- Agent
- Host
- Spike
- Processing

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Scale down:

- Usually left to client
  - Plug into existing viral validation protocol
- Smaller scale is seldom better
  - Surface affects dominate
  - More idiosyncratic
  - Less flexibility in sampling and assay
  - Proportionately larger sampling losses

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Agent:

- Scrapie – many strains
- CJD – many strains
- BSE/v-CJD – one strain

- BSE/vCJD is the relevant strain for this agent
- Otherwise the choice is arbitrary
  - It is not clear that the variation within a disease class is any less than that between disease classes

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Host 1:

- Mouse
- Tg Mice
  - If PrPSc is the agent – it is model of choice, but
  - The most effective tg mice are chimeras; carry the doppel gene; have random and multiple insertions, aberrant expression, in an abnormal context.
  - Tg gene replacement mice by Jean Manson – normal context but not necessarily fast or most susceptible.
- Hamster – convenient, well characterized
- Sheep – endemic scrapie, disappearance of susceptible genotypes
- Cow – should receive more direct study

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Host 2:

- BSE presents very differently in cattle and humans
- Hamster 263K scrapie is clinically more similar to BSE in cattle than BSE in the VM mouse.
- BSE in the VM mouse is clinically more similar to vCJD in humans than BSE in cattle.
- Even the selection of a host is somewhat arbitrary.

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Spike 1: Source of Problem

- TSE infectivity is polydisperse in its physical and chemical properties.
- Subpopulations with differing properties fractionate differently
- Steps that reliably remove most of the infectivity may never be able to remove all of the infectivity
- High titer infectivity is limited to CNS tissues.
- Monodisperse virus preparations like PPV can be introduced anywhere in a process and display the same properties as virus introduced early and surviving to that point.

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Spike 2: Brain derived spikes

- High titer but questionable relevance
- Brain fractions:
  - Microsomes
  - Liposomes
  - DLPCs
  - Caveolar domains
  - Fibrils
  - May behave better but are not necessarily more relevant
- Appropriate if source tissue is brain or cross-contaminated by brain.

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Spike 3: Endogenous Infectivity

- High relevance
- Low titer
  - Have developed methods for accurate titer
- Rodent tissues may not scale well
  - Dura mater is fragile
  - Hearts, kidneys, pituataries, livers, bones, tendons, etc. are small

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Spike 3: Introduction of the Spike

- Intrinsic infectivity
  - Blood - Infectivity in proportion to blood volume of tissue
  - Other intrinsic infectivity
  - Difficult to mimic intrinsic infectivity in solid tissues
    - Dura mater, tendons, hides, bone
    - Failure reduces to testing the spike in the presence of the tissue rather than in the tissue itself.
- Extrinsic infectivity
  - Cross-contamination
    - Brain may be relevant
  - May be the most significant source of infectivity
  - Spiking is the same as contamination

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Other issues

- Sampling
- Sample preparation for titration
- Assay methods
- Control of cross-contamination
- Husbandry logistics and other issues

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Experimental Design 1

- Wherever possible test endogenous infectivity first
  - Highest possible relevance for most tissues
  - Tests removal of intrinsic infectivity
  - Low titer
  - Highly accurate methods for measurement
  - Carry the process forward as far as possible or until there is not possibility of remaining infectivity
  - The claimed efficiency of removal from exogenous spikes can not exceed that obtained for endogenous infectivity

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Example: Cohn Fractionation

- Infectivity from endogenous infected blood could be detected as far as the Fraction IV_1 + IV_4 pellet

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UNPUBLISHED DATA

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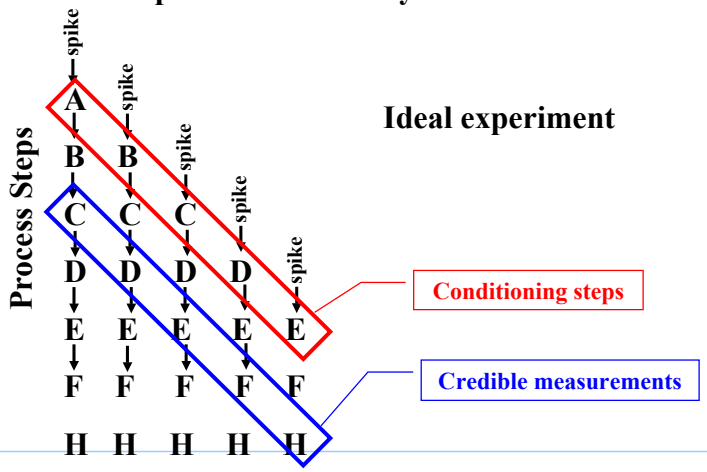
Experimental Design 2: Condition the Spike

- Where spikes are used they should be "conditioned"



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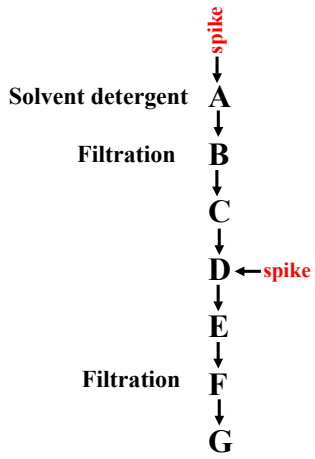
Experimental Design 2: Condition the Spike



Secondary spike at D should be treated with S/D and filtered as in B

Can count removal at F only if spike is pretreated as in A and B

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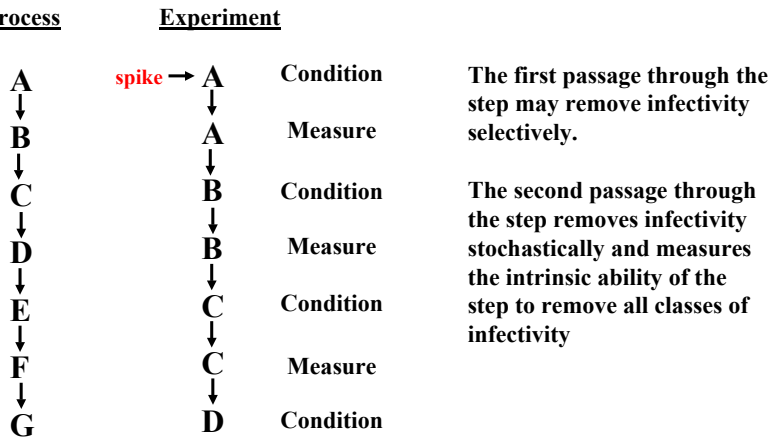
Experimental Design 3: Condition the Spike



The first passage through the step may remove infectivity selectively.

The second passage through the step removes infectivity stochastically and measures the intrinsic ability of the step to remove all classes of infectivity

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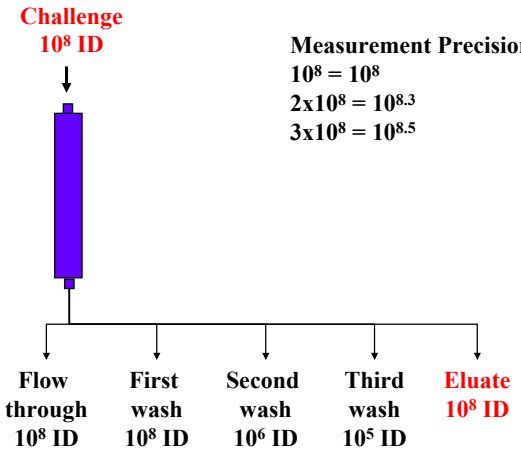
Experimental Design 4: Measure the product stream directly



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Evaluation of Total Clearance 1.

- Endogenous studies take precedence over spikes
- Continuous processing takes precedence over step-wise values
- Great caution should in interpreting the cumulative removal from very similar steps tested step-wise.

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Evaluation of Total Clearance 2.

- The exercise is worthwhile
- High values are better than low ones
- However, the actual values should not be interpreted too literally

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Convergence = Confidence

As data accumulate for multiple agents, spikes, assays, scale downs and animal models, convergence of diverse approaches on the same result provides the greatest security for the outcome.

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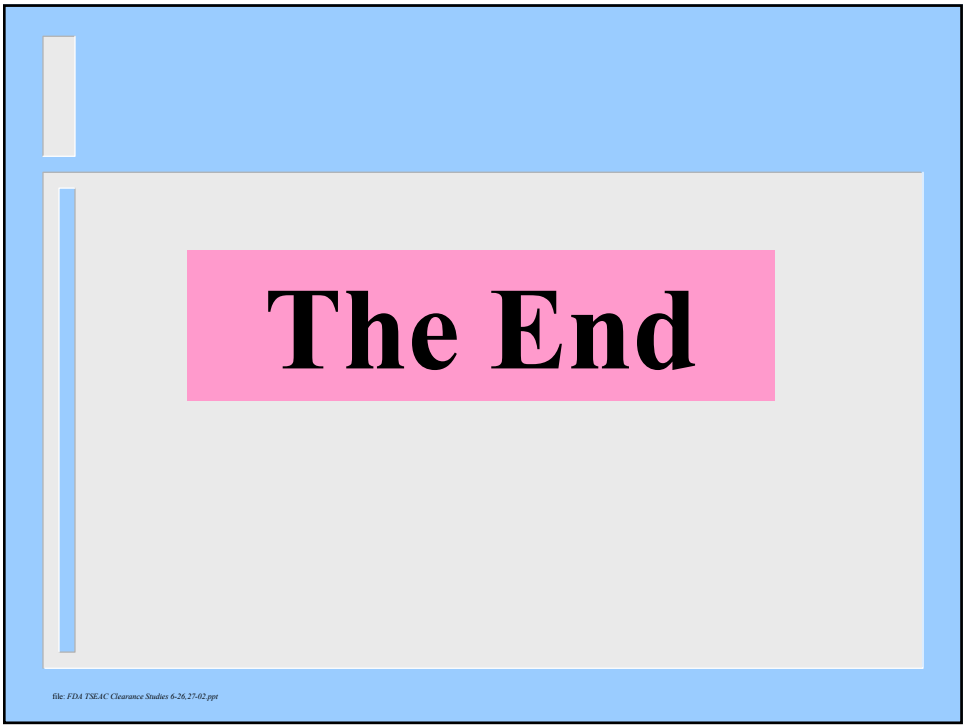
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